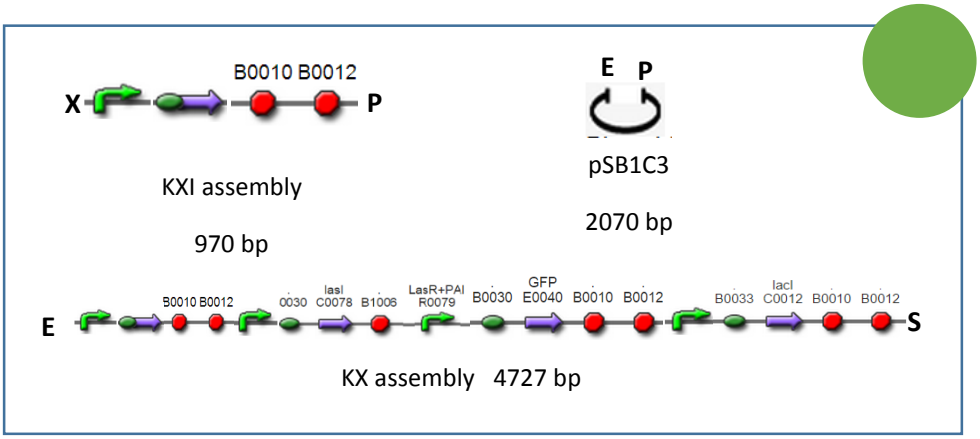
Assembly:

K XIV



1st Day

EXSP Digestion (see **Enzymatic Digestion Protocol**)

Parts	ng/ul	Volume to 2,5 ug (ul)	Buffer x10 (ul)	EcoRI (ul)	XbaI (ul)	SpeI (ul)	PstI (ul)	H_2O to 50ul (ul)
KX assembly	334,0	8,0	5	1	-	1	-	35
KXi assembly	136,0	18,0	5	-	1	-	1	25
pSB1C3	288,0	**3,5 ug** = 12 ul	5	1	-	-	1	31

Repeat this digestion only if you run out of stock

2nd Day

Gel Purification

- See **Kit Wizard SV gel and PCR clean up Promega Protocol**
- Quantify digestion products

Parts	ng/ul	260/280
KX assembly (ES)	27,2	1,75
KXI assembly (XP)	16,1	1,84
pSB1C3 (EP)	31,0	2,05

Obs: 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

Ligation (see **Ligation Protocol**)

Linear Plasmid 50 ng	2 ul	
Insert : Plasmid 3:1 (KX) ; 3:1 (KXI)	KX	KXI
	8 ul	4,5 ul
5x T4 DNA Buffer	4 ul	
T4 DNA ligase 1-5 u	0,5 ul	
H_20 to 20 ul	1 ul	

Obs: To determinate the amount of DNA necessary we used the following equation

$$Insert\ ng = plasmid\ ng \times \frac{insert\ bp}{plasmid\ bp} \times insert{:}\,plasmid\ ratio$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium
- Prepare glycerol 40%

3rd Day

Transformation (see **Transformation Protocol in *Escherichia coli* DH5-α**)

Organism: *E. coli* DH5-α

Selection: Cloranphenicol

4th Day

- Inoculate 3 – 4 colonies overnight in a 6 ml LB with the same antibiotic used in the transformation protocol.
- Incubate overnight at 275rpm/37°C.

5th Day

Miniprep

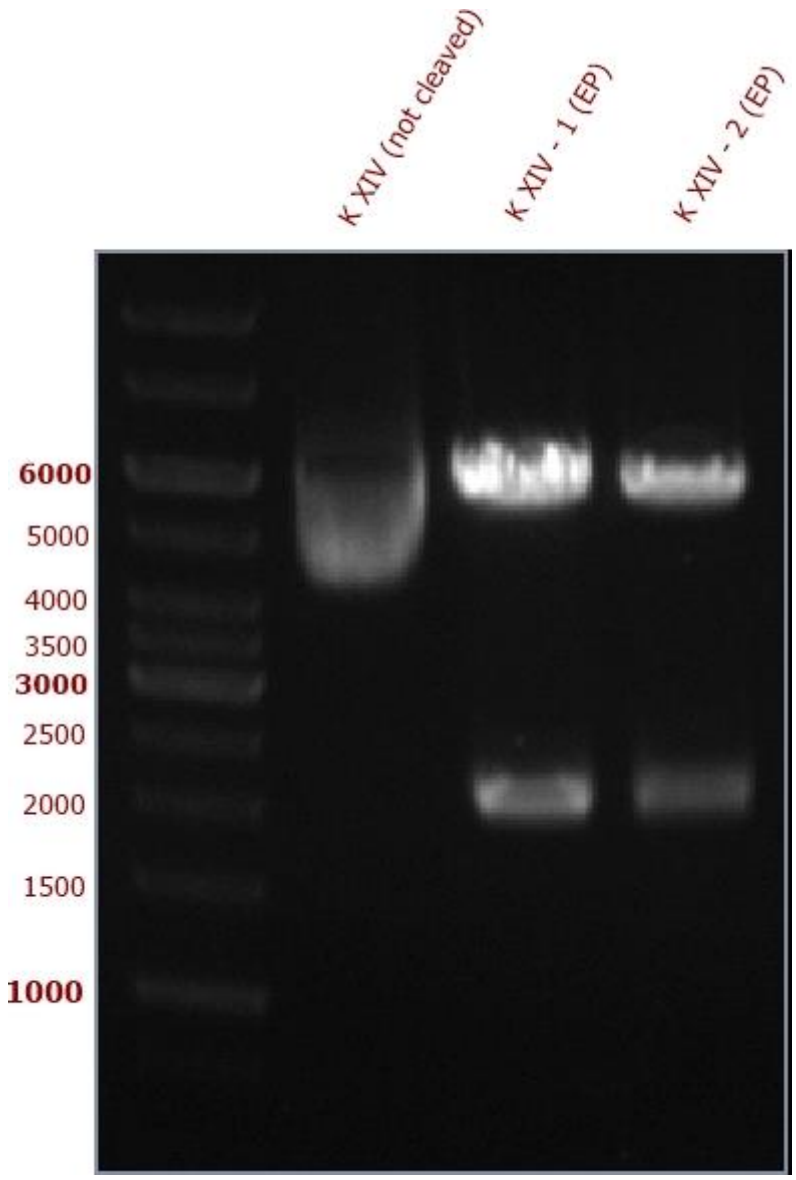
- Prepare **glycerol stock** of the clones (500ul glycerol 40% + 500ul inoculum).
- Extract plasmidial DNA (see **Alkaline Lyses or PureLink Invitrogen Protocol**)
- Run a preliminary electrophoresis gel.
- Quantify DNA samples.

Assembly Confirmation

- EP Digestion (see **Enzymatic Digestion Protocol**)

Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	EcoRI (ul)	PstI (ul)	H_2O to 10ul (ul)
KXIV – 1	2	1	0,5	0,5	6
KXIV – 2	2	1	0,5	0,5	6

- Incubate for 2 hours at 37°C.
- Prepare samples for DNA sequencing.
- Run an electrophoresis analysis of the EP digestion



Size expected	Size in gel
5697 bp	~ 6000 bp